Supplemental Materials

Lactylation at H3K18 drives pathological angiogenesis via metabolic-epigenetic crosstalk in ischemic retinopathy

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Data Online Supplement Figures S1-S7

Data Online Table S1-S4

Supplement Figures and Figure legends

Figure S1

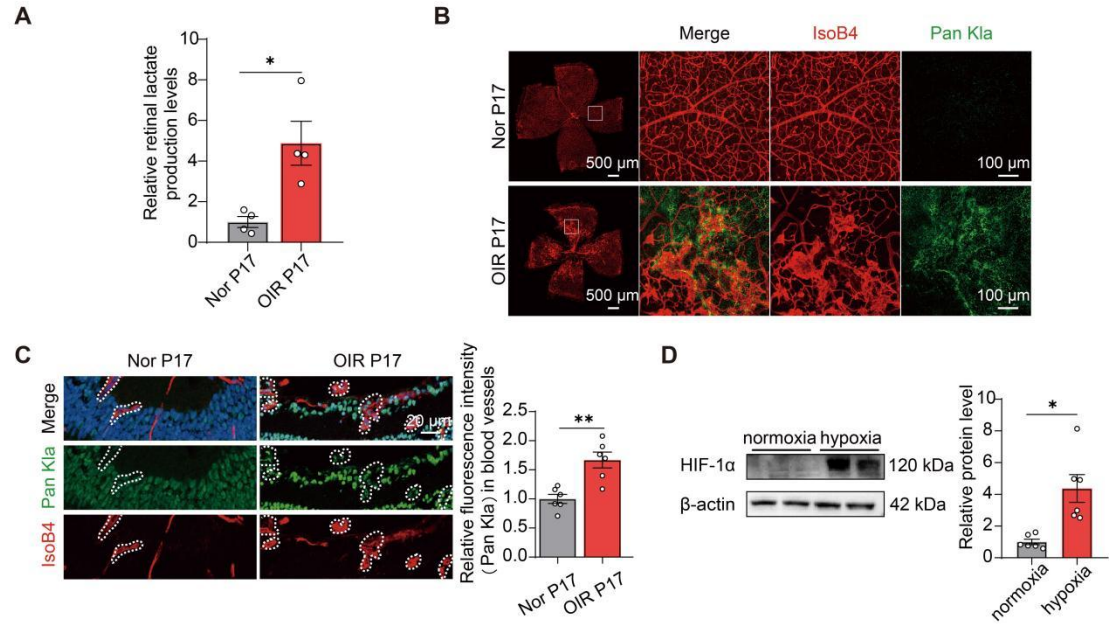

Figure S1. Elevated pan-lactylation levels exhibit significant association with retinal neovascularization in oxygen-induced retinopathy (OIR) models

A: Retinal lactate levels were significantly elevated in oxygen-induced retinopathy (OIR) mice on postnatal day 17 (P17) compared to normoxic controls (Nor P17) (n = 4).

B: Representative retinal whole-mounts from Nor P17 and OIR P17 mice showing costaining with IsoB4 (red) and pan anti-lactyl-lysine antibodies (pan-Kla, green). Scale bars: 500 μm (left panels); 100 μm (right panels).

C: Vascular networks in P17 retinal sections co-labeled with IsoB4 (red) and H3K18la (green) antibodies. Scale bar: 20 μm (n = 6).

D: HIF-1α protein expression was analyzed by western blotting using β-actin as a loading control (n = 6).

Data were analyzed using two-tailed unpaired Student's *t*-test. Data represent mean ± SEM. Significance: **P* < 0.05, ***P* < 0.01.

Figure S2

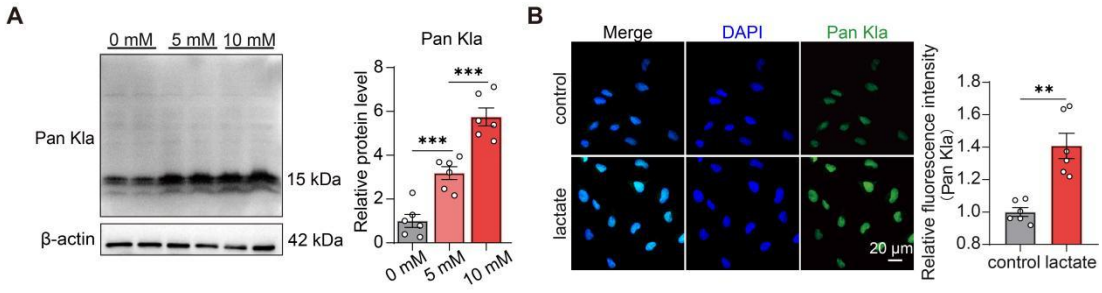

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Figure S2. Lactate administration elevates global lactylation levels

A: HRMECs were treated with lactate (0, 5, and 10 mM) for 24 h. Lysates were immunoblotted with pan anti-lactyl-lysine antibody (pan-Kla), using β-actin as loading control (n = 6).

B: Immunofluorescence staining of HRMECs treated with or without 10 mM lactate, using the pan-Kla antibody (green) and nuclear counterstain DAPI (blue). Scale bar: 20 μm (n = 6).

Data were analyzed using a one-way ANOVA with Tukey's multiple comparison test (A) or a two-tailed unpaired Student's *t*-test (B). Data presented as mean ± SEM (**$P < 0.01$,***$P < 0.001$).

Figure S3

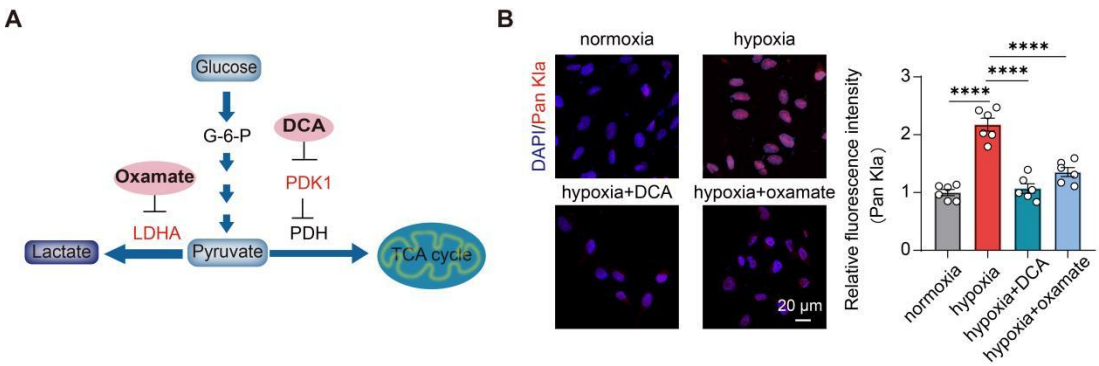

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Figure S3. Lactate inhibition reduces pan-lactylation levels

A: Dichloroacetate (DCA) activates pyruvate dehydrogenase (PDH) by inhibiting pyruvate dehydrogenase kinase 1 (PDK1), thereby shunting the glycolytic end product, pyruvate, into mitochondrial oxidative phosphorylation and reducing lactate production. Oxamate, a structural analog of pyruvate, functions as a competitive inhibitor of lactate dehydrogenase (LDHA). This inhibition suppresses lactate production from pyruvate, thereby disrupting the glycolytic flux.

B: HRMECs were exposed to (i) normoxia (21% O_2), (ii) hypoxia (1% O_2), (iii) hypoxia with 20 mM DCA, or (iv) hypoxia with 20 mM oxamate. Immunostaining with pan anti-lactyl-lysine antibody (pan-Kla, red) and nuclear counterstaining with DAPI (blue). Scale bar: 20 μm (n = 6).

Statistical analysis: One-way ANOVA with Tukey's multiple comparison test. Data are presented as the mean ± SEM (****$P < 0.0001$).

Figure S4

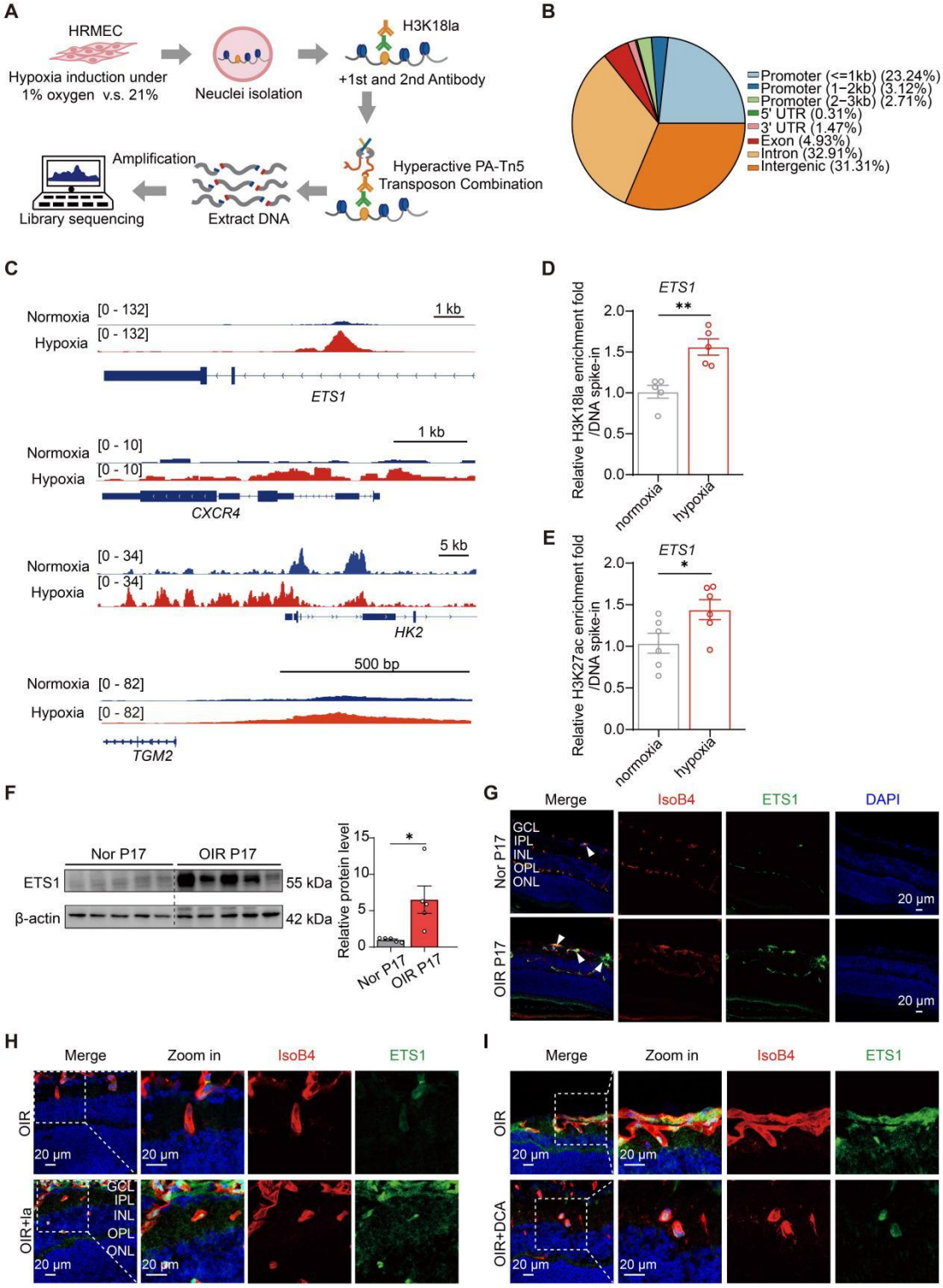
A
HRMEC
Hypoxia induction under
1% oxygen v.s. 21%
Neuclei isolation
H3K18la
+1st and 2nd Antibody
Hyperactive PA-Tn5
Transposon Combination
Amplification
Extract DNA
Library sequencing
B
Promoter (<=1kb) (23.24%)
Promoter (1-2kb) (3.12%)
Promoter (2-3kb) (2.71%)
5' UTR (0.31%)
3' UTR (1.47%)
Exon (4.93%)
Intron (32.91%)
Intergenic (31.31%)
C
Normoxia
Hypoxia
[0 - 132]
1 kb
ETS1
[0 - 10]
CXCR4
[0 - 34]
5 kb
HK2
500 bp
[0 - 82]
TGM2
D
ETS1
**
Relative H3K18la enrichment fold /DNA spike-in
normoxia
hypoxia
E
ETS1
*
Relative H3K27ac enrichment fold /DNA spike-in
F
Nor P17
OIR P17
ETS1
β-actin
55 kDa
42 kDa
Relative protein level
G
Merge
IsoB4
ETS1
DAPI
GCL
IPL
INL
OPL
ONL
20 μm
H
Zoom in
OIR
OIR+Ia
I
OIR+DCA

Figure S4. H3K18 lactylation in endothelial cells may regulate *ETS1* transcription

A: Experimental workflow for CUT&Tag profiling of H3K18 lactylation in HRMECs under normoxic (21% O_2) and hypoxic (1% O_2) conditions.

B: H3K18la peaks demonstrate predominant enrichment in enhancer regions.

C: IGV visualization of H3K18la occupancy at *CXCR4*, *ETS1*, *HK2*, and *TGM2* loci.

D,E: Quantitative CUT&RUN-qPCR confirms the hypoxia-induced co-occupancy of H3K18la (n = 5) and H3K27ac (n = 6) at *ETS1* enhancer (Chr11:128, 467, 536–128, 467, 935).

F: Western blot analysis confirming ETS1upregulation in OIR retinas (n = 5).

G: Immunofluorescence analysis revealed enhanced ETS1 expression colocalized with the IsoB4 vasculature. Scale bar: 20 μm.

H,I: Immunofluorescence quantification reveals elevated ETS1 protein expression in sodium lactate-treated OIR mice (200 mg/kg), whereas dichloroacetate (DCA, 200 mg/kg) attenuated the expression compared with OIR. Scale bar: 20 μm.

Data were analyzed using two-tailed unpaired Student's *t*-test. Data represent mean ± SEM. Significance: $*P < 0.05$, $**P < 0.01$.

Figure S5

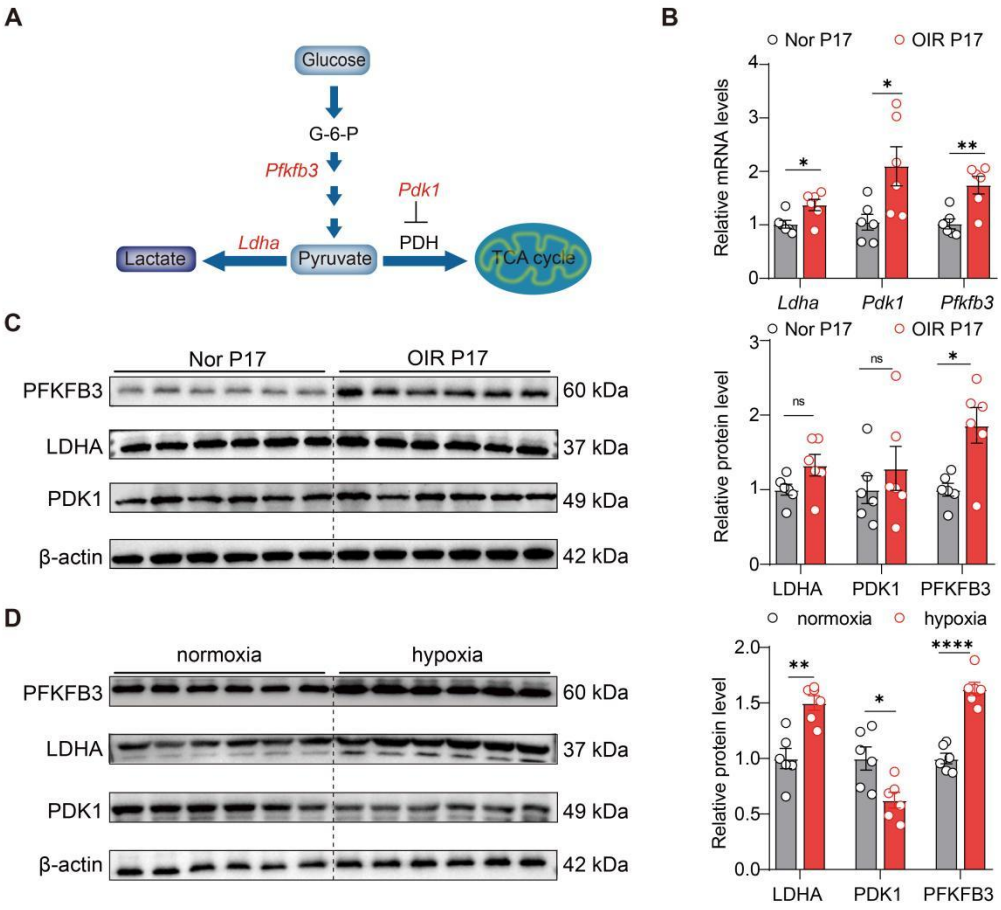

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Figure S5. PFKFB3 expression is elevated in the OIR model and in hypoxic endothelial cells

A,B: Transcriptomic profiling identified robust upregulation of the glycolytic regulators *Pfkfb3*, *Pdk1*, and *Ldha* in OIR retinas compared with those into normoxic controls, independently validated by RT-qPCR (B).

C,D: Western blot demonstrating increased PFKFB3 expression in OIR retinal lysates (C)and hypoxic HRMECs (D).

Data were analyzed using two-tailed unpaired Student's *t*-test. Data represent mean ± SEM. Significance: **P* < 0.05, ***P* < 0.01, *****P* < 0.0001.

Figure S6

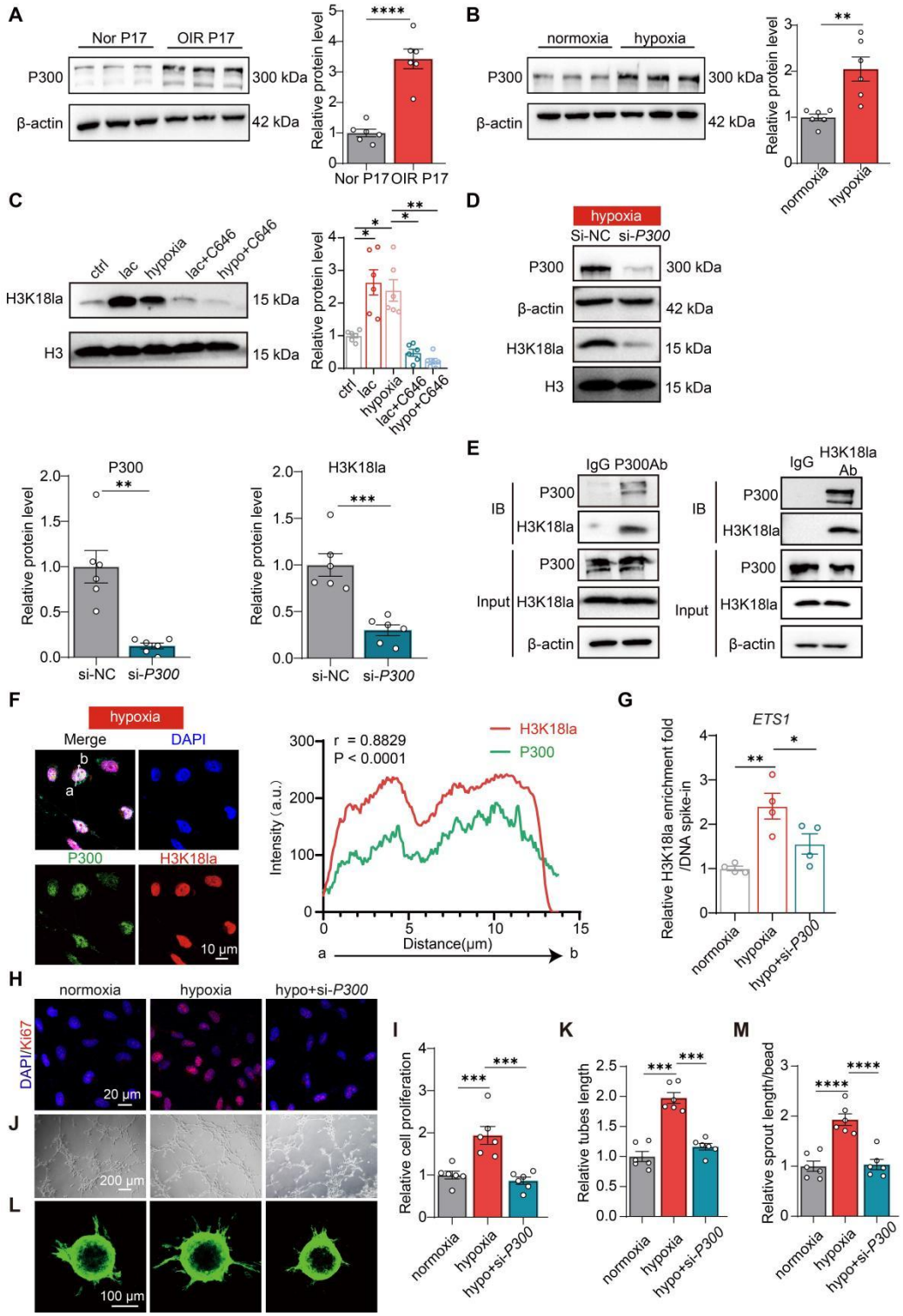
A
Nor P17
OIR P17
P300
β-actin
300 kDa
42 kDa
Relative protein level
****
Nor P17
OIR P17
B
normoxia
hypoxia
P300
β-actin
300 kDa
42 kDa
Relative protein level
**
normoxia
hypoxia
C
ctrl
lac
hypoxia
lac+C646
hypo+C646
H3K18la
H3
15 kDa
15 kDa
Relative protein level
D
hypoxia
Si-NC
si-P300
P300
β-actin
H3K18la
H3
300 kDa
42 kDa
15 kDa
15 kDa
P300
H3K18la
Relative protein level
si-NC
si-P300
E
IgG
P300Ab
IgG
H3K18la Ab
IB
Input
P300
H3K18la
β-actin
F
hypoxia
Merge
DAPI
P300
H3K18la
10 μm
r = 0.8829
P < 0.0001
Intensity (a.u.)
Distance(μm)
a
b
G
ETS1
Relative H3K18la enrichment fold /DNA spike-in
normoxia
hypoxia
hypo+si-P300
H
normoxia
hypoxia
hypo+si-P300
DAPI/Ki67
20 μm
I
Relative cell proliferation
K
Relative tubes length
M
Relative sprout length/bead
J
200 μm
L
100 μm

Figure S6. P300-mediated H3K18 lactylation drives pathological retinal neovascularization

A: P300 expression in retinas of normoxic (Nor P17) and OIR mice at P17. β-actin loading control (n = 6).

B: Hypoxia-induced (1% O_2, 24 h) P300 upregulation in HRMECs (n = 6).

C:H3K18 lactylation in HRMECs under: vehicle control/lactate (10 mM, 24 h)/hypoxia (1% O_2, 24 h)/ lac + C646 (10 μM)/ hypo + C646 (10 μM) (n = 6).

D: *P300* knockdown efficiency and H3K18la suppression using siRNA (50 nM) in hypoxic HRMECs (n = 6).

E: Co-immunoprecipitation validating P300-H3K18la interaction (n = 3).

F: Confocal imaging of P300-H3K18la colocalization:P300 (green)/H3K18la (red). Pearson's r = 0.8829. Scale bar: 10 μm.

G:Genome-wide H3K18la occupancy maps generated by CUT&RUN-qPCR in HRMECs subjected to normoxia (control), hypoxia (1% O_2, 24 h), or hypoxia with *P300* knockdown (n = 4).

H-M: Phenotypic recovery assays after post-*P300* inhibition under hypoxia: H,I: Ki67 proliferation; J,K: Tube formation; L,M: Spheroid sprouting (n = 6; scale bars labeled).

Data represent mean ± SEM. Unpaired two-tailed Student's *t*-tests (A,B, and D) or one-way ANOVA with Tukey's multiple comparison test (C, G, I, K, and M) were performed. Significance: **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

Figure S7

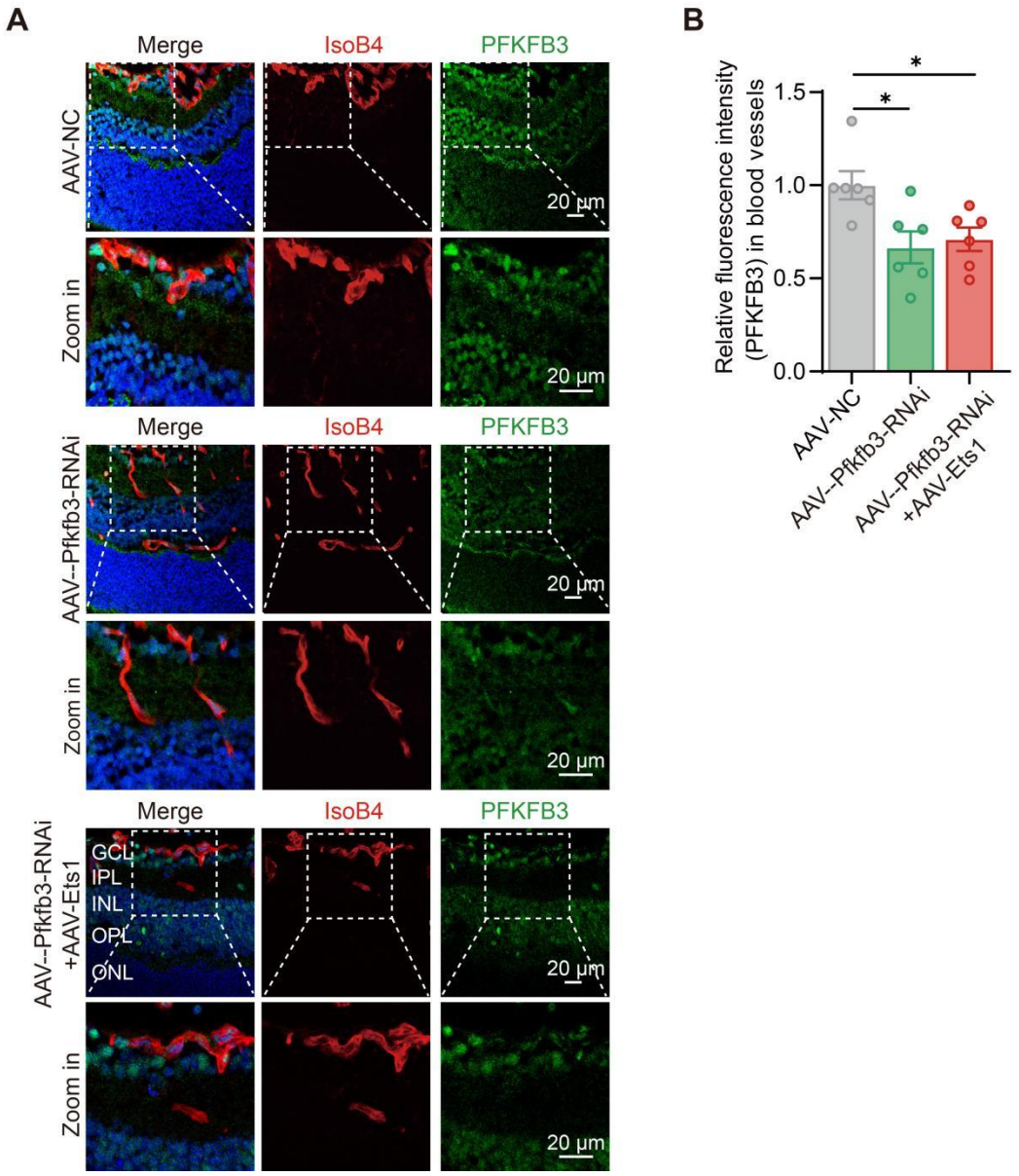
A
Merge
IsoB4
PFKFB3
AAV-NC
Zoom in
AAV--Pfkfb3-RNAi
AAV--Pfkfb3-RNAi
+AAV-Ets1
GCL
IPL
INL
OPL
ONL
20 μm
B
Relative fluorescence intensity
(PFKFB3) in blood vessels
1.5
1.0
0.5
0.0
*
*
AAV-NC
AAV--Pfkfb3-RNAi
AAV--Pfkfb3-RNAi
+AAV-Ets1

Figure S7. PFKFB3 signals are diminished in knockdown retinas

A,B: Quantitative immunofluorescence demonstrating reduced PFKFB3 signal intensity in *Pfkfb3*-knockdown retinas. Scale bar: 20 μm (n = 6).

Supplementary Table 1:The sequences of siRNA

siRNA	Sequence (5'-3')
si*ETS1-a* (Homo sapiens)	CGCUAUACCUCGGAUUACU
si*ETS1-b* (Homo sapiens)	CCACUAUUAACUCCAAGCA
si-*P300* (Homo sapiens)	CCGGUGAACUCUCCUAUAATT
si*PFKFB3* (Homo sapiens)	GAAGAGGATCAGTTGCTATGATT

127 **Supplementary Table 2:Antibodies**

Target antigen	Vendor or Source	Catalog#	Working concentration	Persistent ID/URL
PFKFB3	ABclonal	A3934	1/1000(WB)	https://abclonal.com/catalog-antibodies/PFKFB3RabbitmAb/A3934
PDK1/PDHK1	ABclonal	A0834	1/1000(WB)	https://abclonal.com/catalog-antibodies/PDK1PDHK1RabbitpAb/A0834
LDHA	ABclonal	A1146	1/1000(WB)	https://abclonal.com/catalog-antibodies/LDHARabbitpAb/A1146
Anti-L-Lactyl-Histone H3(Lys18) Rabbit mAb	PTM BIO	PTM-1406RM	1/2000(WB) 1/500(IF)	https://ptmbio.com/products/anti-l-lactyl-histone-h3-lys18-rabbit-mab/PTM-1406RM.htm
Anti-L-Lactyl-Histone H3(Lys14) Rabbit mAb	PTM BIO	PTM-1414	1/1000(WB)	https://ptmbio.com/products/anti-l-lactyl-histone-h3-lys14-rabbit-pab/PTM-1414.htm
Anti-L-Lactyl-Histone H4(Lys5) Rabbit mAb	PTM BIO	PTM-1407RM	1/1000(WB)	https://ptmbio.com/products/anti-l-lactyl-histone-h4-lys5-rabbit-mab/PTM-1407RM.htm
Anti-L-Lactyl-Histone H4(Lys8) Rabbit mAb	PTM BIO	PTM-1415RM	1/1000(WB)	https://ptmbio.com/products/anti-l-lactyl-histone-h4-lys8-rabbit-mab/PTM-1415RM.htm

Anti-L-Lactyl-Histone H4(Lys12) Rabbit mAb	PTM BIO	PTM-1411RM	1/1000(WB)	https://ptmbio.com/products/anti-l-lactyl-histone-h4-lys12-rabbit-mab/PTM-1411RM.htm
Anti-L-Lactyl-Histone H3(Lys18) Rabbit mAb	PTM BIO	PTM-1427RM	1/50 (CUT&Tag)	https://www.ptmbio.com/search/list.htm?searchtype=product&keyword=PTM-1427RM
Anti-Histone H3/HIST3H3 Monoclonal Antibody	Solarbio	K200033M	1/1000(WB)	https://www.solarbio.com/goodsInfo?id=9279
Anti-L-Lactyl Lysine Rabbit mAb	PTM BIO	PTM-1401RM	1/1000(WB) 1/500(IF)	https://ptmbio.com/products/anti-l-lactyllysine-rabbit-mab/PTM-1401RM.htm
Ets-1(C-4)	SANTA CRUZ BIOTECHNOLOGY	sc-55581	1/1000(WB) 1/200(IF)	https://www.scbt.com/zh/p/ets-1-antibody-c-4
Ki67	Abcam	ab16667	1/200(IF)	https://www.abcam.cn/products/primary-antibodies/ki67-antibody-sp6-ab16667.html
DAPI	Abcam	ab104139	IF	https://www.abcam.cn/products/media/mounting-medium-with-dapi-aqueous-fluoroshield-ab104139.html
β-actin	Proteintech	66009-1-Ig	1/1000(WB)	https://www.ptgcn.com/products/Pan-Actin-Antibody-

				66009-1-Ig.htm
Anti-Histone H3 (acetyl K27) - ChIP Grade	Abcam	ab4729	1/50 (CUT&Tag)	https://www.abcam.cn/products/primary-antibodies/histone-h3-acetyl-k27-antibody-chip-grade-ab4729.html
PFKFB3	Abcam	ab181861	1/200(IF)	https://www.abcam.cn/products/primary-antibodies/pfkfb3-antibody-epr12594-ab181861.html

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129 **Supplementary Table3:Primer sequences used in RT-qPCR.**

	Gene	Primer	Primer Sequence (5′-3′)	Length
Homo sapiens	*ETS1*	F	GCCAGTCATCTTTCAACAG	126
		R	CACGGTCCCGCACATAG	
	ACTB	F	AGCACAGAGCCTCGCCTTT	70
		R	ATCATCATCCATGGTGAGCTGG	
	HK2	F	AGGTCCTGATGCGGTTGG	154
		R	CGCTCCTCGCCTTTGTTC	
	CXCR4	F	CCACCAACAGTCAGAGGC	101
		R	AAAGATGAAGTCGGGAATAG	
	TGM2	F	ACAAATCCATCAACCGTTCC	160
		R	GCCAGTTTGTTCAGGTGGTT	

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	Gene	Primer	Primer Sequence (5′-3′)	Length
Mus musculus	*Ets1*	F	GAGATCCTGCAGAAAGAGGATG	178
		R	GGAGCGTCTGATAGGACTCTGT	
	Actb	F	GGCTGTATTCCCCTCCATCG	154
		R	CCAGTTGGTAACAATGCCATGT	
	Pfkfb3	F	GATCTGGGTGCCCGTCGATCACCG	147
		R	CAGTTGAGGTAGCGAGTCAGCTTC	
	Ldha	F	TGTCTCCAGCAAAGACTACTGT	155
		R	GACTGTACTTGACAATGTTGGGA	
	Pdk1	F	AGAGTCTGTCGATCTTCCTCA	96
		R	CGATTCAAGTTCACGTCACAC	

	Tgm2	F	GCTGGACCAACAGGACAATGT	85
		R	CTCTAGGCTGAGACGGTACAG	
	Hk2	F	TGATCGCCTGCTTATTCACGG	112
		R	AACCGCCTAGAAATCTCCAGA	
	Cxcr4	F	GAAGTGGGGTCTGGAGACTAT	125
		R	TTGCCGACTATGCCAGTCAAG	

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132 **Supplementary Table 4:Primer sequences used in CUT&RUN-qPCR assays**

Sites	**Primer Sequence (5′-3′)**
ETS1(Homo sapiens)	Forward primer：GCCCGTGGATGGTCTAAT
	Reverse primer：GATGTGGGTGTCGCTGGT

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